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ASSESSMENT OF GENETIC DIVERSITY WITHIN POPULATIONS OF *NEOTOMA ALBIGULA* (WHITE-THROATED WOODRATS) NATURALLY ASSOCIATED WITH TACARIBE SEROCOMPLEX VIRUSES (FAMILY ARENAVIRIDAE)

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ABSTRACT

Seven microsatellite loci were used to develop multilocus genotypes for 375 individuals of *Neotoma albigula* collected from 32 localities throughout Arizona. Twelve of 32 localities in this study contained arenavirus antibody-positive individuals. Several statistical analyses were used to determine genetic structure, levels of genetic variability, and degree of relatedness in order to assess the effects of the regional gene pool on the presence or absence of arenaviruses. Degree of relatedness was used as a proxy for familial susceptibility within a gene pool. The F_{ST} value (0.110) indicated moderate genetic differentiation among localities. All localities displayed low to moderate levels of genetic diversity in terms of mean observed heterozygosity (0.357–0.787) and mean polymorphic information content (0.256–0.775). Mean relatedness values were slightly negative for all localities, signifying that individuals within localities were less related than individuals taken from a locality at random. Comparison of genetic diversity and relatedness values between antibody-positive and antibody-negative localities indicated no differences among the sites. This suggests that the presence of arenaviruses in certain localities is not associated with variation in genetic diversity or relatedness as detected by these markers.

Key words: genetic variation, microsatellites, *Neotoma albigula*, population genetic structure, probability of identity, relatedness

INTRODUCTION

Neotoma albigula (White-throated Woodrat) is a wide ranging species (Hall 1981; Macêdo and Mares 1988) distributed in southern California, Baja California, southern portions of Utah and Colorado, Arizona, western New Mexico, and northern Mexico (Edwards et al. 2001). Edwards et al. (2001) used DNA sequence

data to split *N. albigula* into two distinct species, *N. albigula* and *N. leucodon* (White-toothed Woodrat). *Neotoma albigula* typically is found in arid areas in a wide variety of habitats including juniper-pinyon woodlands, rocky outcrops, and in association with various cactus species (*Opuntia*; Macêdo and Mares 1988;

citations therein). In Arizona, *N. albigula* often are found in pinyon-juniper woodlands, and in association with cholla and prickly pear cactus (Hoffmeister 1986).

This species is naturally associated with Whitewater Arroyo Virus (WWAV) and other arenaviral species (Fulhorst et al. 1996; Kosoy et al. 1996; Calisher et al. 2001; Abbott et al. 2004), hepatitis E virus (Favorov et al. 2000), *Leishmania mexicana* (the protozoan that causes cutaneous leishmaniasis; Kerr et al. 1999), and hantaviruses (Mantooth et al. 2001), among others. Fulhorst et al. (1996) isolated the WWAV prototype strain AV9310135 from *N. albigula* from Whitewater Arroyo in McKinley County, New Mexico. Subsequently, strains of WWAV or WWA-like viruses have been isolated from *N. macrotis* (Cajimat et al. 2007b; Milazzo et al. 2015), *N. albigula* (Abbott et al. 2004; Milazzo et al. 2008), *N. mexicana* (Cajimat et al. 2008, 2011; Inizán et al. 2010), and *N. micropus* (Fulhorst et al. 2002; Cajimat et al. 2007a, 2011, 2013; Milazzo et al. 2010, 2013).

Abbott et al. (2004) examined 2,434 rodent samples collected from localities throughout Arizona, including 1,250 *N. albigula* samples. Nine percent (112/1,250) of these samples were antibody-positive against WWAV in an indirect fluorescent antibody test; including up to 24 individuals from a single locality. Additionally, samples of *N. albigula* from 12 of 32 collection sites were positive for arenavirus antibodies. This study focused on a subset of 375 samples from the Abbott et al. (2004) study collected from 32 localities. Animals used in this study were found in juniper-pinyon woodlands, montane conifer forests, Sonoran Desert scrub—Arizona upland, Mohave Desert scrub, semi-desert scrub grassland, juniper-pinyon chaparral woodland, and Sonoran Desert scrub—lower Colorado habitats (Abbott et al. 2004). Several individuals also were collected in a citrus orchard. Abbott et al. (2004) reported no statistical association between habitat type and arenavirus prevalence in a given locality.

Because some sites contained antibody-positive individuals, whereas others did not, this study presents the opportunity to examine genetic diversity and relatedness in antibody-positive versus antibody-negative localities. We compared population genetic parameters among sampling localities that were identified by Abbott et al. (2004) as containing antibody-positive individuals to those localities that did not contain antibody-positive individuals to test for effects of the regional gene pool on presence or absence of the virus. The specific objectives of this study were to: 1) examine genetic substructure; 2) examine levels of genetic diversity within and among localities; 3) compare levels of genetic diversity between localities containing antibody-positive individuals to those not containing antibody-positive individuals to determine if the gene pools between the two groups differed; 4) determine degree of genetic relatedness within and among localities; and 5) compare the levels of genetic relatedness between localities that did not contain antibody-positive individuals to those that did contain antibody-positive individuals to determine if the degree of relatedness (as a proxy for familial susceptibility) differed between the two types of sites. To achieve these objectives, multilocus microsatellite genotypes were developed for individuals collected from localities throughout Arizona. Microsatellites have been used to study host population genetics in species that carry diseases such as malaria (Lehmann et al. 1996; Walton et al. 1998; Donnelly et al. 1999, 2001; Pinto et al. 2002; Braginetts et al. 2003; Chen et al. 2004; Tripet et al. 2005), dengue fever (Ravel et al. 2001; Huber et al. 2002; Paupy et al. 2004), and arenaviruses (Méndez-Harclerode et al. 2005, 2007, 2016). Several statistical analyses were used to determine genetic structure, levels of genetic variability, and degree of relatedness in order to assess the effects of the regional gene pool on the presence or absence of arenaviruses.

MATERIALS AND METHODS

Collecting localities and DNA extraction.—Three hundred seventy-five individuals collected from 32 localities in 10 counties throughout Arizona (Table 1, Fig.

1, Appendix) were used in this study. Voucher specimens and tissues for all samples were archived in the Natural Science Research Laboratory at the Museum

Table 1. Locality data for the 364 individuals, collected from 32 localities in Arizona, used in this study. For each locality, site number (Site, corresponding to Fig. 1), specific locality name (Name), latitude/longitude (Lat/Long), number of individuals (N), and presence (P) or absence (A) of arenavirus antibody-positive individuals (AABP) are provided. The numbers in parentheses in this column represent the number of positive individuals used in this study. NA indicates that no latitude/longitude data were gathered for that locality. Antibody status for each individual used in the study is provided in the Appendix.

Site	Name	Lat/Long	N	AABP
1	AZ: Apache Co.; Three Turkey	36°1'44"N/109°24'46"W	1	A
2	AZ: Apache Co.; CDC	NA	1	P (1)
3	AZ: Apache Co.; Saint Johns	34°28'28"N/109°19'18"W	6	A
4	AZ: Navajo Co.; MVP Pig Farm	34°33'28"N/110°4'37"W	12	P (1)
5	AZ: Navajo Co.; Lone Pine Reservoir	34°20'42"N/110°4'53"W	11	A
6	AZ: Navajo Co.; Trick Tank Draw	34°33'43"N/110°46'13"W	10	A
7	AZ: Coconino Co.; Snake Gulch	36°40'18"N/112°22'3"W	2	A
8	AZ: Mohave Co.; Oatman	35°1'56"N/114°16'51"W	15	A
9	AZ: Mohave Co.; Love Camp/Lake Alamo	34°18'26"N/113°33'27"W	20	A
10	AZ: Yavapai Co.; Pine Flat	35°1'12"N/112°49'59"W	18	P (0)
11	AZ: Yavapai Co.; Hillside	34°20'40"N/112°36'59"W	20	A
12	AZ: Yavapai Co.; Wagner	34°25'56"N/112°54'57"W	10	P (6)
13	AZ: Yavapai Co.; Hassayampa	34°20'21"N/112°34'59"W	10	P (7)
14	AZ: Yavapai Co.; Granite Dells Ranch	34°36'55"N/112°23'44"W	19	A
15	AZ: Yavapai Co.; Sycamore Station	34°23'28"N/112°31'1"W	10	P (3)
16	AZ: Yavapai Co.; Horseshoe Ranch	34°15'59" N/112°3'46"W	10	A
17	AZ: Yavapai Co.; Sayer Spring	34°1'0" N/112°39'4"W	20	A
18	AZ: Gila Co.; Barnhardt Trailhead	34°6'8" N/111°22'16"W	10	P (3)
19	AZ: Gila Co.; Windmill Tank	33°57'23" N/111°17'2"W	10	P (4)
20	AZ: Gila Co.; White Cow Mine	33°53'49" N/111°16'57"W	10	P (3)
21	AZ: Gila Co.; Cherry Creek	33°45'49" N/110°48'47"W	7	P (6)
22	AZ: Gila Co.; Gleason Flat	33°46'24" N/110°40'30"W	7	A
23	AZ: Gila Co.; Coon Creek	33°40'59" N/110°51'29"W	7	A
24	AZ: Gila Co.; Sierra Anchas Mountains	NA	2	A
25	AZ: Graham Co.; Warm Springs	33°27'6" N/110°13'33"W	5	A
26	AZ: Graham Co.; Hackberry Creek	33°23'22" N/110°21'30"W	36	P (24)
27	AZ: Graham Co.; Brushy Tank	33°22'23" N/110°18'55"W	20	P (12)
28	AZ: Greenlee Co.; San Francisco River	33°7'30" N/109°16'47"W	6	A
29	AZ: Greenlee Co.; McDowell Road	33°0'21" N/109°14'14"W	10	A
30	AZ: Greenlee Co.; Black Hills	32°5'29" N/109°20'20"W	19	A
31	AZ: Cochise Co.; Chiracahua Mountains	NA	1	A
32	AZ: Yuma Co.; Welton Citrus	32°38'7" N/144°10'4"W	19	A

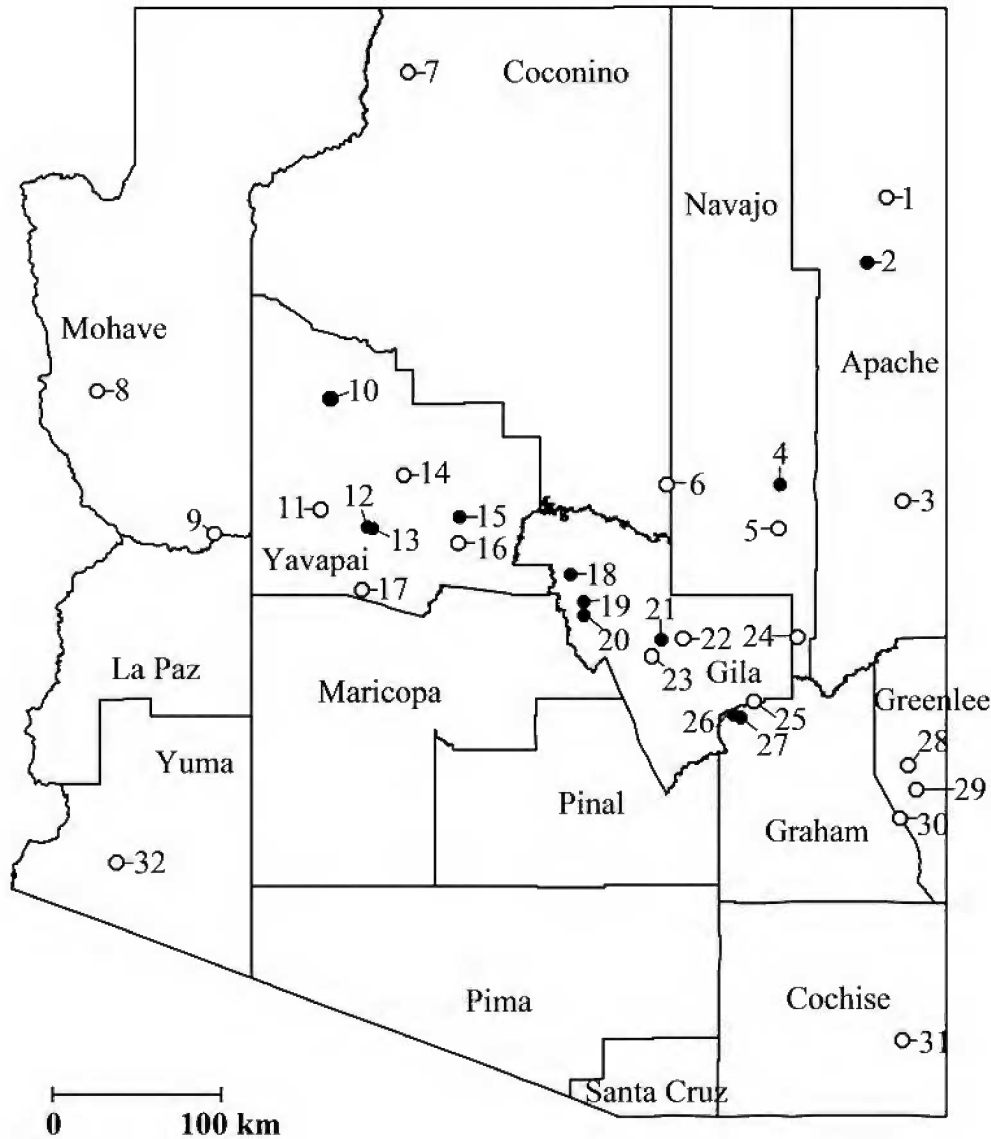


Figure 1. Map of Arizona showing localities where specimens of *Neotoma albigula* were collected. Site numbers correspond to data in Table 1. Black circles represent localities where antibody-positive individuals were collected and open circles represent localities where no antibody-positive individuals were collected.

of Texas Tech University. Because we were interested in gene pool differences between localities and were not directly testing the link between specific alleles and viral infection, we randomly selected individuals from localities without knowledge of their infection status. Where possible, at least 20 individuals were sampled from each locality. Genomic DNA was extracted from approximately 25 mg of liver using a DNeasy Blood and Tissue extraction kit (Qiagen).

Microsatellite analysis.—Twelve microsatellite loci (Table 2) were amplified via the polymerase chain reaction (PCR) using primers developed by Castleberry et al. (2000). PCR amplifications were conducted in 25 μ l volumes containing 1–1.5 μ l genomic DNA, 0.6 μ l 10 pmol each primer, 2.5 μ l 10 X PCR buffer, 1.5–2 μ l 25 mM MgCl₂, 0.75 μ l 10 mM dNTPs, and 0.25 μ l 5U/ μ l Taq. The thermal profile was modified from Castleberry et al. (2000) and consisted of a denaturation

Table 2. Microsatellite loci examined (Castleberry et al. 2000). Product length (PL), number of alleles (A), and sample size (N) for each locus for *N. magister* (Castleberry et al. 2000) and *N. albigula* (this study) are shown. Loci Nma02, Nma03, Nma08, Nma10, and Nma12 were removed from this study due to amplification difficulties.

Locus	<i>N. magister</i>			<i>N. albigula</i>		
	PL	A	N	PL	A	N
Nma01	314-322	6	28	304-348	19	367
Nma02	197-205	4	33	NA	NA	NA
Nma03	180	1	12	NA	NA	NA
Nma04	145-163	7	33	130-185	27	365
Nma05	227-232	4	38	208-223	9	367
Nma06	215-223	5	39	198-275	18	367
Nma08	125-125	7	38	NA	NA	NA
Nma10	186-224	14	39	NA	NA	NA
Nma11	150-160	8	8	142-214	35	365
Nma12	115-127	3	3	NA	NA	NA
Nma14	144-160	7	7	134-176	14	370
Nma15	120-136	10	10	105-149	19	369

and enzyme activation cycle at 94°C (2 min); 35 cycles of 94°C (30 s) denaturation, 55-57°C (30 s) annealing, 72°C (1 min) elongation; followed by a final incubation at 72°C (10 min).

Variation at individual microsatellite loci was examined using an Applied Biosystems 3100-Avant Genetic Analyzer. Reactions included 13.5–14 µl Hi-Di Formamide (Applied Biosystems), 0.5 µl 400HD ROX size standard (Applied Biosystems), and 0.5–1 µl PCR product. Genotypes were scored using GeneMapper version 3.0 software (Applied Biosystems). Alleles that did not amplify above a predetermined peak height (signal strength), were difficult to score, or appeared aberrant were reamplified and rescored.

Statistical analyses.—The program Cervus 2.0 (Marshall et al. 1998) was used to compare alleles to bin files generated from GeneMapper software allowing for determination of typing errors that may have occurred during data entry. Micro-Checker version 2.2.1 (Van Oosterhout et al. 2004) was used to test for presence of null alleles, large allele drop out, and error due to stutter.

A random sample of at least 31 individuals per locus was genotyped twice without knowledge of previous scores. Using these samples, an error rate was calculated by dividing the number of erroneous allele scores at each locus by the total number of allele scores for all individuals for which at least two genotypes existed.

Structure version 2.0 software (Pritchard et al. 2000) was used for assignment tests. Due to large samples sizes, individuals were assigned first to counties, then to localities within counties. Tests of group assignment were based on geographic locality and geographic distance from other collection sites. Under this approach, each locality was considered to represent a separate “population.” The parameters for all assignment tests were: burn-in length = 90,000, MCMC repetitions after the burn-in = 900,000, ancestry model = prior population information, allele frequency model = allele frequencies correlated, and $G = 2$. G calculates the probability of each individual having an ancestor that immigrated from another population. An individual was considered to be assigned correctly if it had at least an 80% probability of being included in

the cluster to which it originally was grouped based on geographic locality.

The program Cervus 2.0 (Marshall et al. 1998) was used to estimate allele frequencies, observed and expected heterozygosity, null allele frequency, and polymorphic information content (PIC-index of variability associated with expected heterozygosity). Probability of identity (PI) was estimated with IDENTITY 1.0 software (Wagner and Sefc 1999) using equations reported by Paetkau et al. (1995). This program also was used to identify identical genotypes among samples and indicate potential parent-offspring combinations. Pairwise and mean relatedness values for each population were estimated with the program Identix 1.1 (Belkhir et al. 2002) using equations developed by Queller and Goodnight (1989). Mean relatedness values were estimated by performing 500 permutations on genotypic data and 95% confidence intervals were calculated after 100 bootstraps across loci.

The program Fstat 2.9.3 (Goudet 2001) was used to estimate deviations from Hardy-Weinberg equilibrium (HWE), linkage disequilibrium, F-statistics (Weir and Cockerham 1984), and R_{ST} (Slatkin 1995; Rousset 1996; Goodman 1997). Sequential Bonferroni corrections (Holm 1979; Rice 1989) were performed on all analyses as a function of this program. The indicative

adjusted nominal level was set at 5%, following traditional tests for significance at the 95% level. For all tests, 1,000 permutations were performed. Arlequin 2.000 software (Schneider et al. 2000) was used to perform an analysis of molecular variance (AMOVA), which allocates percentage of genetic variation at different hierarchical levels, using 10,000 permutations.

Comparisons of genetic diversity and relatedness between antibody-positive and antibody-negative localities were performed using either the comparison-among-groups function of Fstat or t-tests. The program Fstat was used to compare observed heterozygosity, F_{IS} , F_{ST} , relatedness (Hamilton 1971; Pamilo 1984, 1985), and gene diversity (Nei 1987) among the two groups. For each test, 1,000 permutations were performed. T-tests were used to compare expected heterozygosity, PIC, and number of alleles between the two groups. These tests were performed twice. The first group of tests compared all antibody-positive versus antibody-negative localities. The second group of tests compared all antibody-positive localities versus only those localities Abbott et al. (2004) determined to be statistically antibody-negative. This includes sites 5, 6, 8, 9, 11, 14, 16, 17, 22, 23, and 28–32 (Table 1, Fig. 1). Sites 3, 7, 24, and 25 were not included in this group as sample size was determined as a possible limiting factor in the inability to detect the presence of arenavirus antibodies.

RESULTS

Five loci were removed due to amplification difficulties (Table 2). The remaining seven loci were used for all further analyses. Five individuals were removed from the study due to failure to amplify for at least five loci. All other individuals ($n = 370$) were included in population assignment analyses. Twelve data entry errors were detected (12/2,625 entries = 0.457% error rate) and corrected prior to data analysis. Genotype scoring errors were detected at loci Nma05 (4/276 allele calls = 1.449%), Nma11 (2/204 allele calls = 0.980%), and Nma14 (2/138 allele calls = 1.449%). In all instances, two different heterozygotes calls were made for each sample. No evidence for scoring error due to stutter or large allele drop was detected at any locus using Micro-Checker software. The potential presence of null alleles was found at all loci.

All but six individuals were assigned correctly to their respective locality with a probability of 0.800 or higher. Of these six individuals, two were assigned to localities > 320 kilometers from their original collection site and four individuals were assigned to multiple localities with equal probability. These six individuals were removed and the remaining 364 samples were used for all further analyses. Allele calls for the 364 individuals used in the study are provided in the Appendix. Sites 1, 2, and 31 contained a single individual and were not used in any population level analyses. These three individuals were included in the combined assessment of number of alleles, observed and expected heterozygosity, and PIC across all samples reported in Table 3.

Table 3. Summary statistics for each locality and all localities combined. Allele frequencies can be obtained from the senior author upon request. Site numbers correspond to localities in Fig. 1 and Table 1. Three localities (sites 1, 2, and 31) contained a single individual and were not included in any population level analyses, but were included when looking at these statistics across all samples (Total). Number of individuals (N), mean number of alleles (A), mean observed (H_O) and expected (H_E) heterozygosity, HWE p-values over all loci (P), mean polymorphism information content (PIC), F_{IS} , and mean relatedness (R) values are shown below. NA indicates that no test was performed for that statistic. Indicative adjusted nominal level (5%) for HWE was $p < 0.001$ after Bonferroni corrections.

Site	N	A	H_O	H_E	P	PIC	F_{IS}	R
3	6	5.290	0.714	0.720	0.518	0.627	0.008	-0.203
4	12	6.710	0.723	0.776	0.088	0.713	0.071	-0.085
5	11	4.710	0.701	0.710	0.480	0.625	0.013	-0.100
6	10	7.140	0.771	0.738	0.846	0.669	-0.049	-0.110
7	2	1.570	0.357	0.405	0.408	0.256	0.167	-1.000
8	15	6.140	0.619	0.651	0.187	0.602	0.050	-0.070
9	20	8.000	0.607	0.667	0.025	0.625	0.092	-0.050
10	18	6.860	0.611	0.673	0.019	0.633	0.096	-0.060
11	20	7.140	0.729	0.698	0.879	0.648	-0.045	-0.053
12	10	6.290	0.671	0.707	0.247	0.639	0.053	-0.112
13	10	6.860	0.686	0.723	0.213	0.659	0.055	-0.113
14	19	7.000	0.616	0.658	0.090	0.620	0.064	-0.059
15	10	6.290	0.557	0.644	0.015	0.587	0.141	-0.107
16	10	5.290	0.600	0.609	0.457	0.552	0.016	-0.114
17	20	8.570	0.664	0.682	0.289	0.644	0.026	-0.056
18	10	6.140	0.714	0.653	0.962	0.587	-0.099	-0.106
19	10	5.710	0.698	0.673	0.761	0.608	-0.040	-0.111
20	10	5.570	0.643	0.643	0.559	0.574	0.000	-0.105
21	7	6.000	0.653	0.710	0.141	0.626	0.086	-0.164
22	7	6.860	0.673	0.830	0.001	0.741	0.202	-0.157
23	7	6.570	0.775	0.776	0.662	0.684	-0.013	-0.151
24	2	2.860	0.786	0.833	0.394	0.515	0.100	-1.000
25	5	5.570	0.686	0.794	0.052	0.672	0.150	-0.250
26	36	9.430	0.770	0.802	0.087	0.766	0.040	-0.028
27	20	10.140	0.779	0.820	0.098	0.775	0.052	-0.051
28	6	5.430	0.724	0.762	0.235	0.657	0.053	-0.222
29	10	7.710	0.757	0.798	0.198	0.726	0.055	-0.109
30	19	9.430	0.782	0.813	0.175	0.767	0.040	-0.053
32	19	7.570	0.787	0.769	0.722	0.720	-0.023	-0.061
Total	364	19.430	0.697	0.811	NA	0.797	NA	NA

Mean number of alleles ranged from 1.570 (site 7) to 10.140 (site 27) within the localities (Table 3). Mean observed and expected heterozygosities, and PIC are reported in Table 3. Two individuals with identical genotypes were detected. These genotypes were confirmed, as was the identity of the original samples, and both samples were left in all analyses. Eight potential parent-offspring groupings were detected. PI was $1.340e^{-9}$ (1 chance in 746 million of randomly selecting two individuals with the same genotype). Pairwise relatedness values ranged from slightly negative to highly positive within sites. Mean relatedness values were negative for all sites (Table 3).

The significance level for tests of HWE within sites was set at $p < 0.001$ after Bonferroni corrections. Across all loci, sites were in HWE (Table 3). The adjusted Bonferroni p-value for disequilibrium was 0.002. No genotypic disequilibrium was detected ($P > 0.007$ for all pairwise comparisons). F_{IS} ranged from -0.099 (site 18) to 0.202 (site 22) within sites (Table 3). F-statistic values among sites, with 95% confidence intervals in parentheses, were as follows: $F_{IT} = 0.145$ (0.084, 0.223), $F_{ST} = 0.110$ (0.074, 0.160), and $F_{IS} = 0.040$ (-0.009, 0.102). Pairwise differentiation comparisons are shown in Table 4. Three estimators of R_{ST} were calculated among sites: weighted = 0.133, Goodman = 0.141, and unweighted = 0.140. Results

of the AMOVA indicated that 10.630% of the variation was among sites, 3.050% of the variation was among individuals within sites, and 86.320% of the variation was within individuals.

Comparisons between antibody-positive and all antibody-negative localities resulted in no significant differences between the parameters. The results from Fstat analyses compared at the 5% nominal level were as follows: $P(H_O) = 0.905$, $P(F_{IS}) = 0.536$, $P(F_{ST}) = 0.856$, $P(\text{Relc, corrected relatedness}) = 0.536$, and $P(H_S, \text{gene diversity}) = 0.418$. The results of the t-tests compared at the 5% nominal level were as follows: $t = -0.194$, $df = 27$, $P > 0.500$ when comparing H_E ; $t = 0.618$, $df = 27$, $P > 0.500$ when comparing PIC; and $t = 0.998$, $df = 25$, $P > 0.300$ when comparing number of alleles. Comparisons between the two groups after sites 3, 7, 24, and 25 were removed resulted in no significant differences between the groups. The results from Fstat analyses compared at the 5% nominal level were as follows: $P(H_O) = 0.969$, $P(F_{IS}) = 0.706$, $P(F_{ST}) = 0.976$, $P(\text{Relc, corrected relatedness}) = 0.707$, and $P(H_S, \text{gene diversity}) = 0.855$. The results of the t-tests compared at the 5% nominal level were as follows: $t = -0.556$, $df = 39$, $P > 0.500$ when comparing H_E ; $t = -0.407$, $df = 20$, $P > 0.500$ when comparing PIC; and $t = -0.106$, $df = 20$, $P > 0.500$ when comparing number of alleles.

DISCUSSION

Genetic structure.—Comparison of the F_{ST} value (0.110) to guidelines provided by Wright (1978) indicated moderate genetic differentiation between sites. Pairwise differentiation values indicated 231 significant comparisons (Table 4); although there was no discernable pattern among the differentiation values. For example, site 3, located in Apache County, was not significantly different from any other site with the exceptions of sites 26 and 27, located in Graham County, and site 30, located in Greenlee County. Conversely, site 4 had significant pairwise differentiation values when compared to all sites except 22, 23, 25, and 28. Interestingly, site 4 contained individuals that were antibody-positive, whereas all individuals from sites 22, 23, 25, and 28 were antibody-negative. As might be expected, site 32, which is geographically isolated from all other populations, was significantly different

from all other sites with the exception of site 3. Overall, those sites located on the geographic perimeter of the sampling area (i.e., site 32) tended to be genetically distinct from other populations, whereas those sites that were clustered together (i.e., sites 18-24) tended to be genetically similar to one another, but distinct from locations outside the cluster. In addition to geographic locality, sample size as it relates to potential genetic variation might also have played a role. For example, site 3 only contained six individuals. If the six individuals selected had common genotypes, the genetic diversity within the population would be lower and they would not differ genetically from other populations.

Genetic variation.—All sites possessed low to moderate levels of genetic variability based on values for observed heterozygosity and PIC (Table 3). The PI

(1 chance in 746 million of randomly selecting two individuals with the same genotype) also was low compared to other species of *Neotoma* (Haynie et al. 2007, 2009). Small sample sizes for most of the populations were reflected in the low levels of variation. Adding to the low levels of variability was the dominance of a single or a few alleles at several loci, especially locus Nma14. Most allele calls at this locus (73.78%) represented a single allele, thus most individuals were fixed for a single allele at this locus thereby decreasing genetic variation. Results of the AMOVA indicated that most of the genetic variation was within sites. However, the amount of variation among sites (~11%) supported the findings that there is some genetic structure and several sites were genetically different from one another.

Relatedness.—Pairwise relatedness values ranged from slightly negative to highly positive within sites, with mean relatedness values being negative for all sites (Table 3). Negative relatedness values indicate pairs of individuals in these sites are less related to one another than are pairs of individuals taken from a population at random. Despite negative mean relatedness values, some individuals within sites did show some degree of relatedness. Relatedness values ranged from as low as 0.002 (site 6; indicative of a distant cousin relationship) to as high as 0.756 (site 26; indicative of parent-offspring or sibling relationship). Eight potential parent-offspring groupings were detected, one each within sites 5, 6, 9, and 19 and two each within sites 10 and 26.

Mode of transmission in arenaviruses still is in question, although some clues have arisen. Fulhorst et al. (2001), in a laboratory experiment, determined that viral transmission could occur both vertically (parent to offspring) and horizontally (between contemporary individuals). Calisher et al. (2001), in a study of wild woodrats, determined that transmission between rodents was through direct contact. Abbott et al. (2004) determined that there was no association between being antibody-positive and aggressive behavior between individuals, based on skin wounds, for the individuals used in this study. They also determined that there was no relationship between age or sex classes and being antibody-positive. Abbott et al. (2004) concluded that vertical transmission is an important process in virus transmission in natural populations. If vertical transmission is important, it can be predicted that localities

with a high degree of arenavirus prevalence will have a high degree of relatedness, suggesting familial susceptibility. Preliminary assessment of the correlation between relatedness and antibody status does not indicate a link between the two (data not shown), although that does not mean that a link is not present, simply that it was not detected using these markers.

The lack of closely related individuals within these sites is not surprising. Similar patterns of relatedness values have been found in *N. macrotis* (Matocq and Lacey 2004; Haynie et al. 2007), *N. fuscipes* (Haynie et al. 2007), and *N. stephensi* (Haynie et al. 2009). In addition, Matocq and Lacey (2004) found that females, typically thought to be closely related to neighboring females, actually were not closely related nor philopatric. This pattern has not been studied in *N. albigula*, but it may explain low relatedness values. Additionally, the sampling strategy of this study was not aimed at collecting all neighboring individuals and therefore may have affected relatedness values.

Arenavirus association.—Of the 32 localities studied, 12 contained individuals positive for arenavirus antibodies. Most of the positive localities were located centrally within the state (Fig. 1). However, there was no clear pattern as to the presence or absence of the virus based on geographic location. Localities that contained antibody-positive individuals were relatively close to sites that did not contain antibody-positive individuals. There are several possible explanations for the distribution of the virus in the study area. First, there may be a link between habitat type and virus susceptibility. However, Abbott et al. (2004) tested this possibility and found no such relationship within the localities studied. Second, there may be a genetic link to susceptibility. Based on our analyses, the levels of genetic differentiation and genetic diversity between localities did not appear to be affected by the presence or absence of arenavirus-positive individuals at the site. Although comparisons of antibody-positive and antibody-negative localities indicated that there was no difference in genetic variation and relatedness between the two, the markers used in this study are not directly tied to virus susceptibility. Additionally, we were not assessing genetic differences between antibody-positive and antibody-negative individuals, but rather differences between the gene pools of sites containing positive individuals to sites that did not. Further

analyses and the utilization of markers directly tied to immunity and virus uptake in the host are warranted.

Additional explanations exist for the distribution of viruses seen in this study, beyond those tested. The presence of antibody-positive individuals in some populations and not in others could be the result of a founder effect. Virus-positive individuals could have moved into certain localities and not others, thus bringing the virus to certain populations. This idea currently is beyond the scope of this study, but remains a possible explanation for the distribution of the virus. It also is possible that some virus-positive localities that were distantly separated from other virus-positive localities (e.g., Sites 2 and 4) represent viral refugia. Again, this explanation is beyond the scope of this study, but warrants further investigation. Finally, it may be that more populations contained antibody-positive specimens and these individuals simply were not collected or the virus was not detected. Abbott et al. (2004) determined that

sample size could have been a limiting factor at four sites (sites 3, 7, 24, and 25) for which no virus antibody was detected. However, 15 sites (sites 5, 6, 8, 9, 11, 14, 16, 17, 22, 23, and 28-32) were determined to be statistically antibody-negative.

Conclusions.—*Neotoma albigula* is a widespread and readily abundant species which has the possibility of easily coming into contact with humans, especially in the central portion of Arizona. These samples and this species warrant further study due to the fact that there may be multiple arenavirus strains associated with *N. albigula* (Milazzo et al. 2015). Mark-recapture studies or other sampling methods that would allow for the development of a pedigree within these populations may help address the question pertaining to the route of viral transmission. This study provides the basic population genetic groundwork for this species and should serve as a stepping-stone for further investigations.

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APPENDIX

Individual genotypes for the 364 *Neotoma albigula* samples used in all analyses for this study. Site numbers correspond to localities in Fig. 1 and Table 1. Individuals are identified by Museum of Texas Tech University catalog number (TTU-M). Antibody status is provided for each individual (N = negative, POS = positive). Loci (Nma01, Nma04, Nma05, Nma06, Nma11, Nma14, and Nma15) correspond to Table 2. * indicates missing data for that locus.

Site	TTU-M	Antibody Status	Nma01	Nma04	Nma05	Nma06	Nma11	Nma14	Nma15							
1	99945	N	322	328	135	164	214	216	206	206	163	182	137	137	131	137
2	115438	POS	324	326	130	130	*	*	202	202	165	165	151	168	117	135
3	106370	N	324	330	137	170	214	214	206	206	184	186	134	137	123	127
3	106371	N	320	326	168	172	*	*	212	218	165	186	134	139	117	129
3	106372	N	324	324	160	168	214	214	208	210	167	184	134	137	125	133
3	106373	N	316	322	170	174	214	214	208	214	178	184	134	134	129	133
3	100793	N	316	322	168	172	214	214	208	216	182	184	137	137	127	131
3	100669	N	306	306	170	174	214	214	206	208	*	*	137	137	125	131
4	97572	N	312	318	137	137	220	220	208	216	165	178	134	134	133	137
4	97573	POS	322	324	156	170	214	220	206	210	165	169	137	137	129	133
4	97574	N	316	322	137	166	210	214	202	214	160	175	137	137	133	133
4	106647	N	320	324	137	156	210	214	210	210	165	180	137	137	129	133
4	106648	N	314	314	139	139	216	216	206	210	178	178	137	137	129	131
4	106649	N	316	324	137	141	214	214	202	204	169	175	134	137	127	133
4	106751	N	314	314	137	160	214	214	216	222	178	197	137	137	127	129
4	106752	N	312	320	139	166	210	214	210	214	160	178	137	137	133	137
4	106753	N	322	324	141	172	210	216	202	214	178	186	137	137	123	129
4	106754	N	*	*	166	172	210	214	208	210	160	160	134	137	125	127
4	106755	N	322	322	141	172	210	220	202	202	167	193	134	137	127	131
4	106756	N	312	314	166	172	210	220	202	208	160	175	137	137	131	135
5	100790	N	322	322	145	156	214	216	208	210	165	188	134	137	125	139
5	106636	N	322	324	141	156	214	216	206	210	165	173	134	137	125	139
5	106637	N	324	324	141	166	210	216	208	214	173	188	137	137	120	120
5	106638	N	322	324	153	166	216	216	202	214	173	188	137	137	120	120
5	106639	N	322	322	139	141	214	220	208	210	188	199	134	137	133	139

Site	TTU-M	Antibody Status	Nma01	Nma04	Nma05	Nma06	Nma11	Nma14	Nma15					
5	106641	N	328	141	210	214	206	214	173	173	134	134	139	139
5	106642	N	324	153	216	216	208	208	173	175	137	137	120	125
5	106643	N	320	141	210	216	208	208	167	173	134	137	117	120
5	106758	N	320	166	210	216	208	214	175	188	137	137	120	125
5	106759	N	324	141	214	214	206	208	173	188	134	137	139	139
5	106760	N	312	139	214	220	208	210	173	199	134	137	133	139
6	106655	N	314	141	210	214	210	216	165	188	137	137	123	133
6	106656	N	314	168	214	220	206	214	169	184	137	137	127	133
6	106657	N	312	137	214	216	210	218	190	190	137	162	120	137
6	106658	N	332	145	208	214	202	216	150	190	137	137	125	129
6	106659	N	320	164	214	220	208	210	186	190	137	137	129	139
6	106761	N	314	168	214	214	208	210	190	190	137	162	133	137
6	106762	N	316	158	214	214	*	*	160	197	137	162	125	125
6	106763	N	320	162	210	214	210	214	165	165	137	137	123	125
6	106764	N	320	149	214	214	208	216	163	165	137	162	133	137
6	106766	N	314	149	214	220	206	208	163	190	137	162	135	137
7	100621	N	*	*	220	220	218	225	165	165	155	155	133	149
7	100622	N	*	*	220	220	218	226	178	178	155	168	133	141
8	106615	N	318	168	214	214	202	218	184	197	137	137	123	143
8	106616	N	320	166	210	214	218	220	165	193	137	137	133	135
8	106617	N	320	168	214	216	202	202	160	160	137	137	123	129
8	106618	N	320	170	210	210	212	214	160	188	137	137	120	139
8	106619	N	320	168	210	216	202	202	160	193	137	137	117	129
8	106614	N	324	172	214	214	202	214	188	197	137	137	111	143
8	106620	N	320	162	214	216	202	216	165	193	137	137	113	135
8	106621	N	320	132	214	216	202	202	160	165	137	137	123	123
8	106622	N	320	170	216	216	202	202	160	160	137	137	123	123
8	106623	N	322	158	214	214	202	202	188	190	137	137	129	135
8	106624	N	320	156	214	216	202	216	160	193	137	137	113	117
8	106625	N	320	143	214	216	202	218	184	193	137	137	111	120
8	106626	N	322	172	214	214	202	202	163	197	137	137	129	143

Site	TTU-M	Antibody Status	Nma01	Nma04	Nma05	Nma06	Nma11	Nma14	Nma15
8	106627	N	322	156	214	202	184	190	120
8	106628	N	318	168	214	202	188	193	117
9	88213	N	318	160	210	202	173	193	125
9	88214	N	316	141	210	202	188	190	123
9	88215	N	322	147	214	216	169	188	135
9	88216	N	318	162	208	202	188	201	129
9	88217	N	322	158	210	202	186	188	127
9	88218	N	316	141	210	212	188	190	123
9	88219	N	322	160	210	202	173	188	123
9	88220	N	322	139	216	202	180	188	129
9	88221	N	318	158	210	202	184	188	143
9	88222	N	318	153	210	202	186	193	135
9	88223	N	318	168	214	202	188	195	125
9	88224	N	316	139	210	214	188	188	123
9	88225	N	318	139	214	202	180	209	135
9	88226	N	320	139	214	216	169	188	135
9	88227	N	318	137	210	216	163	188	135
9	88228	N	318	168	214	202	188	195	123
9	88229	N	320	160	214	202	180	188	135
9	88247	N	322	158	214	202	188	190	135
9	88248	N	322	151	214	214	180	190	143
9	88249	N	320	156	210	202	188	195	133
10	111588	N	322	160	214	202	160	193	131
10	111589	N	318	162	210	202	186	190	129
10	111594	N	324	158	214	202	169	190	131
10	111595	N	324	158	214	202	169	190	123
10	111596	N	322	164	214	202	160	169	131
10	111597	N	324	160	214	202	160	193	129
10	111598	N	322	166	216	212	188	188	135
10	111599	N	314	143	208	202	163	169	137
10	111600	N	322	164	214	202	169	193	131

Site	TTU-M	Antibody Status	Nma01	Nma04	Nma05	Nma06	Nma11	Nma14	Nma15
10	111631	N	324	164	214	202	186	193	120
10	111601	N	320	168	210	214	165	188	127
10	111602	N	322	158	210	202	169	197	123
10	115405	N	322	139	214	202	197	197	123
10	111603	N	322	162	214	218	184	201	127
10	111605	N	316	166	216	202	188	197	*
10	111606	N	318	164	210	202	184	201	129
10	111607	N	322	162	214	212	184	184	127
10	111608	N	318	164	208	202	184	184	129
11	97696	N	320	166	214	202	195	203	120
11	97697	N	320	149	214	202	160	188	127
11	97698	N	314	153	214	202	188	203	131
11	97699	N	322	158	214	202	163	193	133
11	106834	N	318	162	210	202	160	188	127
11	106835	N	320	158	214	202	188	197	123
11	106836	N	320	158	174	202	188	193	133
11	106837	N	324	156	214	202	184	186	123
11	106833	N	320	135	214	202	180	195	120
11	106838	N	314	158	214	202	188	195	123
11	106839	N	322	162	210	202	188	203	127
11	106840	N	324	158	170	212	163	197	125
11	106841	N	314	166	170	202	184	184	120
11	106842	N	314	156	162	202	195	197	120
11	106843	N	320	149	158	216	188	193	127
11	106844	N	318	135	158	202	182	205	123
11	106845	N	320	158	162	212	186	193	120
11	106846	N	314	164	166	202	193	197	123
11	106914	N	324	156	160	212	186	197	123
11	106915	N	320	158	172	202	186	188	129
12	97808	N	316	162	166	212	186	186	127
12	97809	POS	320	160	172	202	182	188	127

Site	TTU-M	Antibody Status	Nma01	Nma04	Nma05	Nma06	Nma11	Nma14	Nma15						
12	97810	POS	314	139	166	210	214	202	208	180	197	137	137	125	127
12	97812	POS	314	166	183	210	210	202	208	188	195	137	137	125	127
12	97813	POS	318	320	170	214	220	202	202	160	188	137	137	125	127
12	97814	POS	318	320	162	210	210	202	212	188	197	137	139	117	125
12	97815	POS	320	328	164	210	216	212	212	186	197	137	141	123	125
12	97631	N	316	316	139	214	214	208	212	163	180	137	137	127	127
12	97632	N	320	322	166	214	214	202	212	160	163	137	137	125	131
12	97636	N	316	326	164	214	214	212	216	180	186	137	137	123	133
13	97675	POS	314	316	166	210	216	202	202	212	214	137	137	125	125
13	97676	POS	314	318	160	214	214	202	208	163	188	137	137	117	125
13	97677	POS	314	320	174	214	214	202	212	184	195	137	137	117	129
13	97678	POS	314	320	170	214	216	212	212	163	197	137	141	129	129
13	97679	POS	320	320	141	214	216	202	212	190	193	137	137	129	129
13	97680	N	314	322	166	214	216	216	216	178	197	137	137	125	129
13	97681	POS	316	320	164	210	214	202	222	165	182	137	137	125	125
13	97682	POS	316	326	164	210	214	210	216	171	197	137	137	120	127
13	97683	N	320	324	170	208	214	202	222	171	188	137	141	129	131
13	97695	N	318	318	170	208	214	202	202	186	193	137	137	117	117
14	88146	N	322	326	164	210	214	202	202	188	190	137	137	125	133
14	88147	N	318	320	156	214	214	202	202	163	193	137	137	123	123
14	88148	N	318	324	164	214	214	202	214	163	193	137	137	123	123
14	88149	N	322	322	162	210	214	202	202	188	193	137	137	123	125
14	88150	N	322	322	168	214	216	202	208	160	193	137	137	111	125
14	88151	N	322	326	168	214	214	202	216	182	182	137	137	123	131
14	88152	N	320	322	153	210	216	202	212	160	163	137	137	123	137
14	88153	N	312	312	166	210	216	202	222	163	186	137	137	117	125
14	88154	N	322	326	158	214	220	202	218	163	197	137	137	123	127
14	88155	N	312	320	156	208	214	202	216	163	163	137	137	123	123
14	88156	N	324	324	162	208	214	202	222	160	182	137	137	123	135
14	88157	N	312	312	164	208	214	202	218	160	193	137	137	123	125
14	88158	N	326	326	158	214	216	202	202	182	197	137	137	125	125

Site	TTU-M	Antibody Status	Nima01	Nima04	Nima05	Nima06	Nima11	Nima14	Nima15						
14	88159	N	320	158	208	214	208	214	182	193	137	137	120	125	
14	88161	N	322	324	162	170	214	216	202	180	190	137	137	123	133
14	88162	N	322	322	166	166	212	214	202	186	190	137	137	127	137
14	88163	N	320	320	162	168	208	208	202	160	197	137	137	120	133
14	88164	N	320	320	162	166	208	214	202	163	182	137	137	125	129
14	88165	N	318	326	168	168	208	214	202	160	197	137	137	123	123
15	97734	N	322	322	143	158	214	216	202	160	199	137	137	127	133
15	97735	N	316	322	164	170	210	210	202	163	171	137	137	129	133
15	97736	N	322	322	170	172	214	214	202	195	197	137	137	123	135
15	97737	N	318	318	166	174	214	214	216	160	199	137	137	127	139
15	97738	N	316	322	162	166	214	216	202	160	199	137	137	117	123
15	97739	N	320	322	156	160	214	214	202	193	195	137	137	123	129
15	97740	N	318	318	143	170	214	214	202	160	199	137	137	123	127
15	97784	POS	314	314	162	164	214	214	202	175	197	137	137	120	133
15	97785	POS	318	318	162	168	214	214	202	186	188	137	137	123	123
15	97786	POS	320	322	166	172	216	216	202	182	182	137	137	123	127
16	99955	N	320	326	153	168	214	214	202	165	199	137	137	125	125
16	99956	N	324	324	156	162	208	214	202	195	199	137	137	123	125
16	99957	N	320	324	143	158	214	214	208	193	199	137	137	109	120
16	99958	N	320	322	172	172	214	214	202	163	163	137	137	125	125
16	99959	N	320	324	153	162	214	214	202	160	199	137	137	125	125
16	99960	N	320	324	156	174	208	214	212	195	199	137	137	120	125
16	99961	N	320	326	154	168	216	216	202	165	199	137	137	117	125
16	99963	N	320	330	170	181	214	214	214	160	163	137	137	123	125
16	99964	N	320	326	153	170	214	216	202	165	193	137	137	125	125
16	99965	N	320	330	141	181	214	216	202	160	163	137	137	125	131
17	111705	N	322	326	164	168	214	216	216	160	167	137	137	131	135
17	111707	N	322	324	158	170	214	214	202	160	188	137	137	125	127
17	111708	N	314	326	141	170	214	214	202	195	201	137	137	123	137
17	111709	N	316	322	158	164	210	216	202	160	197	137	137	123	127

Site	TTU-M	Antibody Status	Nima01	Nma04	Nma05	Nma06	Nima11	Nima14	Nima15							
17	111710	N	314	322	164	174	208	216	212	220	160	169	137	137	117	123
17	111711	N	314	322	158	160	210	210	202	202	171	193	137	137	123	141
17	111712	N	314	322	160	168	210	216	202	202	188	193	137	137	125	141
17	111713	N	324	328	158	164	216	216	202	216	182	197	137	137	123	125
17	111714	N	316	326	139	158	210	216	202	212	188	197	137	137	117	131
17	111715	N	322	322	164	164	214	214	202	208	188	199	137	137	123	129
17	111716	N	320	324	145	158	214	214	202	216	165	188	137	137	127	131
17	111717	N	320	324	139	164	216	216	202	202	182	193	137	137	123	125
17	111718	N	320	322	168	168	214	214	202	202	160	160	137	137	123	139
17	111719	N	322	322	162	168	214	220	202	202	186	199	137	139	125	141
17	111720	N	322	326	139	170	214	216	202	220	188	195	137	137	117	123
17	111721	N	320	320	158	168	210	210	202	202	171	171	137	137	120	123
17	111722	N	322	324	139	153	214	218	202	202	195	199	137	137	123	127
17	111723	N	320	324	143	158	210	214	202	214	160	173	137	137	129	139
17	111724	N	324	324	153	170	214	216	202	212	160	160	137	137	127	129
17	111725	N	320	326	147	160	210	214	202	202	160	160	137	137	125	129
18	97138	POS	322	326	141	156	210	214	202	208	173	180	137	137	129	133
18	97139	POS	324	326	145	174	210	216	208	208	178	193	137	137	120	123
18	97140	POS	318	326	141	156	210	210	208	208	*	*	137	137	123	133
18	97030	N	322	324	139	177	210	214	208	226	163	180	137	137	125	131
18	97031	N	316	322	139	177	210	214	208	226	180	184	137	137	120	123
18	97032	N	316	322	168	170	210	214	208	226	175	180	137	137	120	123
18	97033	N	324	326	156	177	210	214	208	208	167	193	137	137	127	133
18	97034	N	326	335	141	164	210	214	202	202	167	173	137	137	123	133
18	97035	N	322	326	135	177	214	214	208	208	180	193	137	137	120	133
18	97036	N	320	320	166	177	214	214	208	208	163	182	137	137	137	143
19	97159	POS	322	324	166	168	214	214	202	222	178	180	137	168	127	131
19	97160	POS	320	335	166	174	214	214	202	210	173	180	137	137	120	123
19	97161	N	320	326	139	170	210	214	202	208	148	173	137	137	117	127
19	97162	N	322	322	139	170	210	210	202	208	175	178	137	137	151	135
19	97163	POS	322	326	174	174	214	216	202	212	173	195	137	137	123	131

Site	TTU-M	Antibody Status	Nma01	Nma04	Nma05	Nma06	Nma11	Nma14	Nma15						
19	97164	POS	322	324	153	174	214	220	202	148	175	137	137	109	131
19	97165	N	322	322	162	162	214	214	202	148	173	137	137	120	123
19	97166	N	322	324	166	172	210	214	202	*	*	137	137	123	127
19	97167	N	322	324	145	162	210	214	222	173	175	137	137	120	131
19	97168	N	322	322	166	168	214	214	202	180	180	137	168	127	131
20	97144	POS	322	336	143	168	214	214	202	171	180	137	137	129	129
20	97145	POS	320	322	168	170	214	214	202	171	188	137	137	123	127
20	97146	POS	314	320	168	170	214	214	202	171	175	137	137	123	127
20	97049	N	320	322	143	170	210	214	208	175	193	137	137	120	127
20	97050	N	320	322	168	170	214	214	202	165	190	137	137	127	137
20	97053	N	322	324	166	174	210	214	208	175	180	137	137	120	131
20	97055	N	324	324	170	174	210	214	202	175	195	137	137	127	127
20	97056	N	322	324	170	170	210	214	208	212	188	137	137	129	143
20	97057	N	320	324	160	170	210	214	212	148	184	137	137	123	123
20	97058	N	324	324	158	170	214	214	202	184	190	137	151	120	125
21	88387	POS	326	326	141	168	210	214	208	214	165	137	137	129	131
21	88388	POS	320	324	162	162	210	220	202	163	175	137	137	109	113
21	88389	POS	322	322	162	181	210	214	208	212	169	137	137	125	125
21	88390	POS	320	324	162	168	210	214	202	190	199	137	137	127	131
21	88391	POS	310	320	139	153	214	214	208	165	175	137	139	109	120
21	88392	POS	316	332	132	172	214	214	202	175	205	137	137	120	127
21	88393	N	322	322	162	174	210	214	208	169	178	137	137	129	129
22	97037	N	314	330	149	172	214	214	208	180	180	134	139	131	131
22	97038	N	330	330	168	172	210	220	208	167	178	137	139	131	139
22	97039	N	314	314	147	166	220	221	208	156	158	137	137	105	139
22	97040	N	314	320	143	156	210	216	204	158	160	137	155	125	127
22	97041	N	322	330	143	168	210	223	208	180	190	137	137	127	131
22	97042	N	310	318	164	168	216	216	216	182	197	134	134	123	123
22	97043	N	322	322	141	149	220	220	208	163	195	137	137	120	123
23	88402	N	318	320	168	170	214	220	202	178	180	137	137	123	131
23	88403	N	316	320	156	172	214	220	202	160	186	137	137	127	133

Site	TTU-M	Antibody Status	Nma01	Nma04	Nma05	Nma06	Nma11	Nma14	Nma15							
23	88404	N	320	145	177	214	214	202	226	160	173	137	160	109	123	
23	99864	N	320	337	166	168	210	220	202	210	158	178	137	137	123	129
23	99865	N	320	320	153	170	214	220	208	214	156	184	137	137	109	123
23	99866	N	322	322	141	181	220	220	208	226	158	178	137	162	120	125
23	99867	N	318	318	160	170	210	221	210	222	178	178	137	137	127	131
24	115440	N	322	324	143	168	214	214	208	208	*	*	137	137	117	120
24	115441	N	316	322	158	160	210	220	202	225	175	178	137	162	117	129
25	99907	N	320	328	153	164	216	216	208	208	146	171	137	157	127	135
25	99908	N	320	322	149	174	220	220	208	210	190	193	134	137	133	145
25	99909	N	316	316	149	168	210	216	210	210	167	182	134	137	120	129
25	99910	N	314	314	160	170	210	220	208	208	165	186	137	157	120	143
25	99911	N	318	318	139	145	220	220	208	208	175	186	137	137	120	135
26	99871	N	318	318	139	145	220	220	208	208	175	186	137	137	120	135
26	99872	POS	320	332	145	151	214	220	208	210	180	186	137	139	120	135
26	99873	POS	314	326	158	170	220	220	208	220	156	167	137	139	111	135
26	99874	N	314	314	141	158	216	216	208	220	156	182	134	137	111	135
26	99875	POS	314	318	164	170	214	220	220	220	150	158	168	168	127	131
26	99876	POS	318	318	132	164	220	220	214	220	154	158	134	168	127	135
26	99877	POS	318	330	157	157	218	218	214	220	180	195	139	168	120	133
26	99878	POS	312	314	166	177	220	220	208	208	163	186	137	139	131	137
26	99879	POS	314	322	137	145	216	220	210	212	150	182	137	168	120	135
26	99880	POS	318	318	145	151	220	220	214	220	160	160	160	168	125	127
26	99881	N	314	316	158	172	220	220	208	208	154	156	137	168	135	135
26	99882	POS	320	330	145	151	216	218	210	212	158	180	139	168	135	137
26	99883	N	322	330	168	270	220	220	208	220	160	182	137	139	125	127
26	99884	N	320	330	149	170	220	220	208	210	160	186	137	137	111	135
26	99885	POS	320	320	143	166	220	220	212	214	180	180	139	139	125	135
26	99886	POS	316	320	145	179	210	216	208	210	150	160	134	137	120	135
26	99887	POS	314	330	137	145	220	220	212	218	180	180	134	134	120	127
26	99888	POS	316	326	145	170	210	220	210	220	150	182	137	137	120	125
26	99889	POS	314	320	141	166	220	220	208	212	180	180	139	168	127	135

Site	TTU-M	Antibody Status	Nma01	Nma04	Nma05	Nma06	Nma11	Nma14	Nma15
26	99890	POS	314	141	220	208	180	168	127
26	99891	POS	314	151	218	208	180	139	125
26	99892	POS	314	137	214	216	156	134	127
26	99893	POS	314	145	220	212	160	139	127
26	99894	N	316	170	216	210	173	139	125
26	99895	POS	316	132	220	208	178	137	135
26	99896	POS	314	141	220	212	154	168	127
26	99897	N	320	141	220	212	150	168	131
26	99898	POS	314	145	218	218	163	160	120
26	99899	POS	320	151	220	210	165	139	133
26	99900	POS	322	145	216	210	150	168	120
26	99901	N	316	168	216	210	167	139	111
26	99902	N	314	158	216	208	156	168	111
26	99903	POS	314	139	220	212	156	137	111
26	99904	N	330	153	218	210	163	160	131
26	99905	N	320	139	220	210	156	168	111
26	99906	N	314	145	216	208	175	168	127
27	99834	POS	314	145	210	210	186	137	120
27	99835	N	314	166	220	208	182	137	131
27	99836	N	312	147	214	214	154	168	141
27	99837	POS	318	132	214	214	165	157	131
27	99838	POS	314	166	216	214	156	160	120
27	99839	N	314	164	214	210	146	139	120
27	99840	N	314	139	214	208	144	139	127
27	99841	POS	320	151	216	214	169	137	120
27	99842	N	314	139	214	208	175	139	127
27	99843	N	314	139	214	208	154	137	127
27	99844	POS	314	164	216	208	154	157	127
27	99845	POS	314	139	214	210	160	137	127
27	99846	POS	330	139	216	208	169	166	105
27	99847	POS	324	151	220	208	146	137	117

Site	TTU-M	Antibody Status	Nma01	Nma04	Nma05	Nma06	Nma11	Nma14	Nma15							
27	99848	POS	320	330	156	164	210	221	208	216	152	182	134	137	120	131
27	99849	POS	312	316	156	170	214	220	208	216	154	184	134	157	129	131
27	99850	N	314	320	170	172	221	221	210	220	154	186	134	160	131	131
27	99851	N	314	320	158	158	210	216	208	214	173	193	137	137	120	127
27	99852	POS	314	328	158	166	214	216	208	214	169	173	137	137	120	125
27	99853	POS	314	316	158	166	214	214	208	214	163	175	137	137	117	120
28	106520	N	320	328	162	164	218	218	210	225	154	197	134	134	123	131
28	106521	N	320	322	156	162	210	216	210	218	154	154	137	139	129	133
28	106522	N	326	332	162	168	216	216	210	225	154	182	134	134	123	131
28	106770	N	332	332	*	*	216	216	*	*	182	182	134	134	127	131
28	106771	N	308	316	*	*	*	*	204	214	163	167	134	137	131	141
28	106772	N	322	322	162	170	216	220	214	225	158	175	134	134	111	123
29	106503	N	326	326	158	174	214	216	208	208	163	193	137	137	127	141
29	106504	N	304	326	147	158	214	220	208	208	160	173	134	137	117	131
29	106505	N	330	337	156	156	216	216	208	218	163	165	137	137	123	127
29	106506	N	320	326	145	158	216	216	210	220	156	158	134	137	127	133
29	106507	N	304	324	135	147	214	218	208	214	193	193	134	134	123	145
29	106508	N	326	326	147	174	214	216	208	210	193	203	134	137	131	137
29	106509	N	308	314	156	170	218	218	202	216	160	201	134	137	127	129
29	106513	N	320	326	147	147	216	216	214	216	163	188	134	137	117	117
29	106514	N	316	326	158	164	214	214	208	208	160	163	137	168	123	141
29	106515	N	320	330	168	170	216	220	208	216	142	160	137	139	111	129
30	97175	N	324	324	137	168	216	218	210	210	175	199	134	170	131	141
30	97176	N	320	324	137	147	223	223	210	210	156	207	134	134	129	137
30	97177	N	316	320	147	172	216	216	214	218	160	160	134	139	117	125
30	97178	N	320	320	170	172	216	216	202	208	163	175	134	139	129	137
30	97179	N	320	334	145	156	216	223	210	210	156	167	134	139	123	133
30	97180	N	324	330	170	172	216	216	210	212	160	163	134	139	120	133
30	97181	N	320	330	170	174	218	218	208	208	160	165	134	168	123	129
30	97183	N	316	335	162	174	220	220	210	210	163	188	134	155	129	129
30	97184	N	322	335	158	172	220	220	210	210	188	195	134	155	123	129

Site	TTU-M	Antibody Status	Nma01	Nma04	Nma05	Nma06	Nma11	Nma14	Nma15							
30	97185	N	314	145	170	216	220	208	214	160	167	134	137	120	129	
30	97186	N	322	324	174	177	216	220	210	214	188	195	134	134	129	133
30	97187	N	316	332	172	172	220	220	210	275	160	165	139	166	123	137
30	130461	N	320	330	162	170	216	220	202	218	163	163	137	139	127	129
30	130462	N	316	324	162	172	214	218	208	210	154	163	134	137	129	133
30	130463	N	316	320	135	147	210	218	210	214	158	167	134	137	120	141
30	130464	N	320	320	135	147	220	220	208	210	156	171	137	137	120	129
30	130465	N	316	322	161	172	214	220	208	210	163	188	134	134	123	127
30	130466	N	322	324	172	174	218	220	210	210	154	195	134	137	129	133
30	130467	N	304	326	139	145	216	216	202	208	158	175	137	139	120	120
31	89870	N	312	326	166	179	216	216	210	210	171	175	137	137	113	131
32	97646	N	318	318	174	179	210	221	198	202	156	195	137	168	133	135
32	97647	N	318	318	147	174	214	216	202	218	160	190	137	168	127	133
32	97648	N	318	324	135	166	221	221	202	204	144	167	137	137	123	123
32	97649	N	318	318	166	172	216	220	204	218	144	193	137	168	123	135
32	97650	N	312	318	174	185	216	220	204	216	144	190	137	170	133	135
32	97651	N	312	318	147	147	210	221	202	204	167	195	137	168	127	129
32	97653	N	318	320	135	174	208	216	204	214	167	195	137	168	123	127
32	97654	N	322	330	147	174	208	221	212	218	160	195	137	137	123	133
32	97655	N	318	318	166	166	208	210	198	204	160	195	137	137	123	131
32	97656	N	318	324	147	185	208	210	202	202	*	*	137	137	123	135
32	97657	N	304	320	162	164	208	220	202	202	190	195	137	168	127	129
32	97658	N	316	330	147	174	208	208	202	212	167	195	137	137	123	135
32	97659	N	322	322	166	174	216	223	202	222	144	190	137	176	127	133
32	97660	N	304	318	158	172	208	221	198	202	190	195	137	137	123	127
32	97661	N	304	322	166	166	208	208	204	222	156	167	137	168	127	133
32	97662	N	320	324	*	*	220	220	*	*	144	160	137	168	135	139
32	97663	N	312	322	174	174	220	220	202	222	190	190	137	168	127	133
32	97664	N	318	318	166	174	210	216	198	214	156	167	137	137	127	127
32	97665	N	318	326	157	166	208	214	202	222	188	190	137	137	127	129